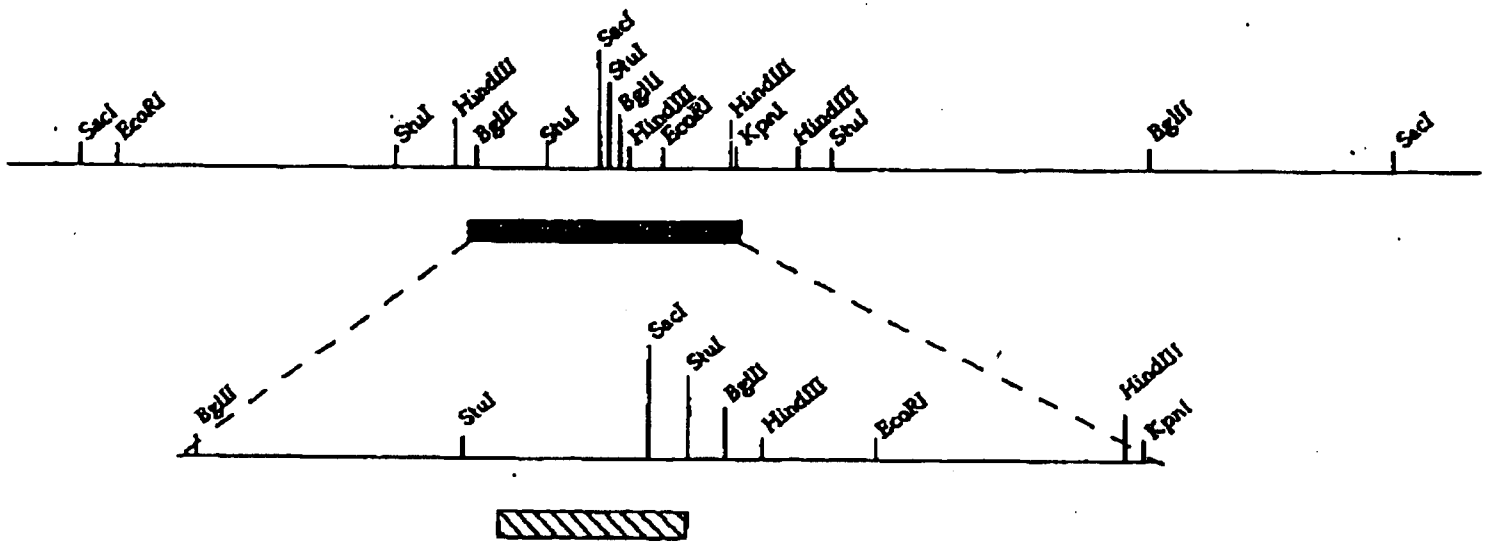
Partial Restriction Map of DNaseB2 and it adjacent region
in Streptococcus pyogenes



2 kb for the expanded map

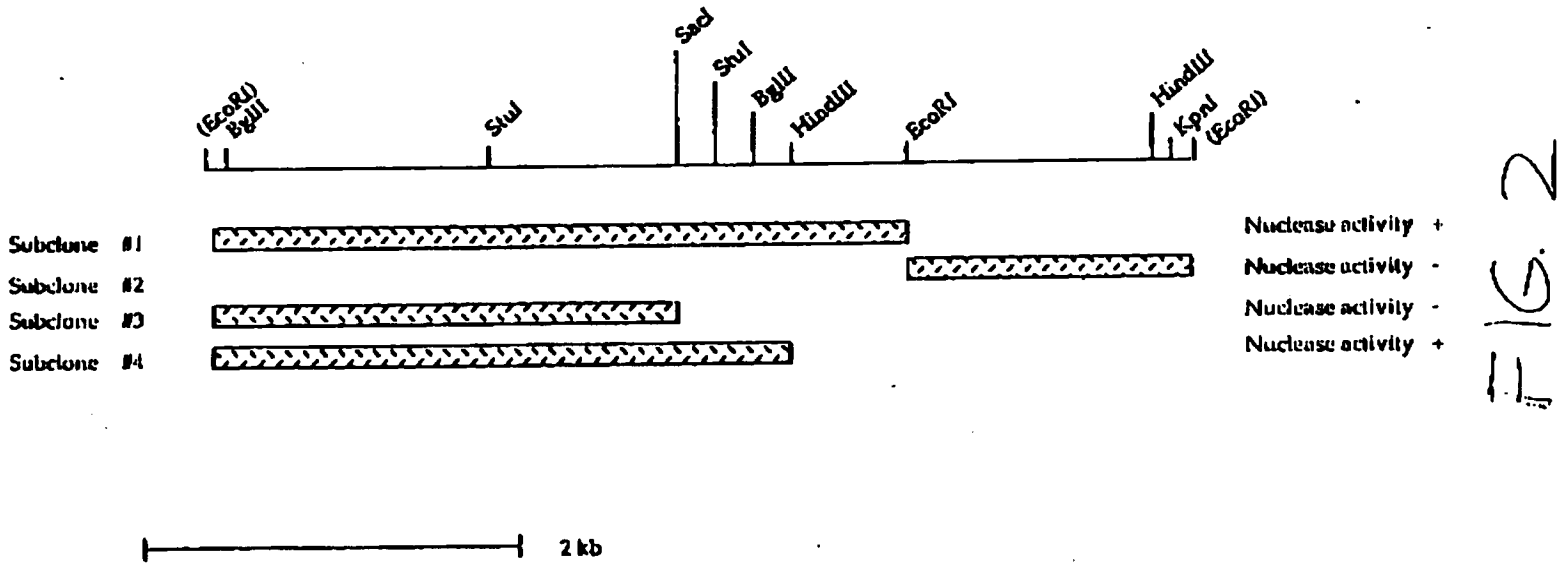
2 kb for the top restriction map

Region cloned in DNaseB2-6

DNaseB2 gene

FIG. 1

Subclones of DNaseB2 and its ability to produce active nuclease.



12
6
11

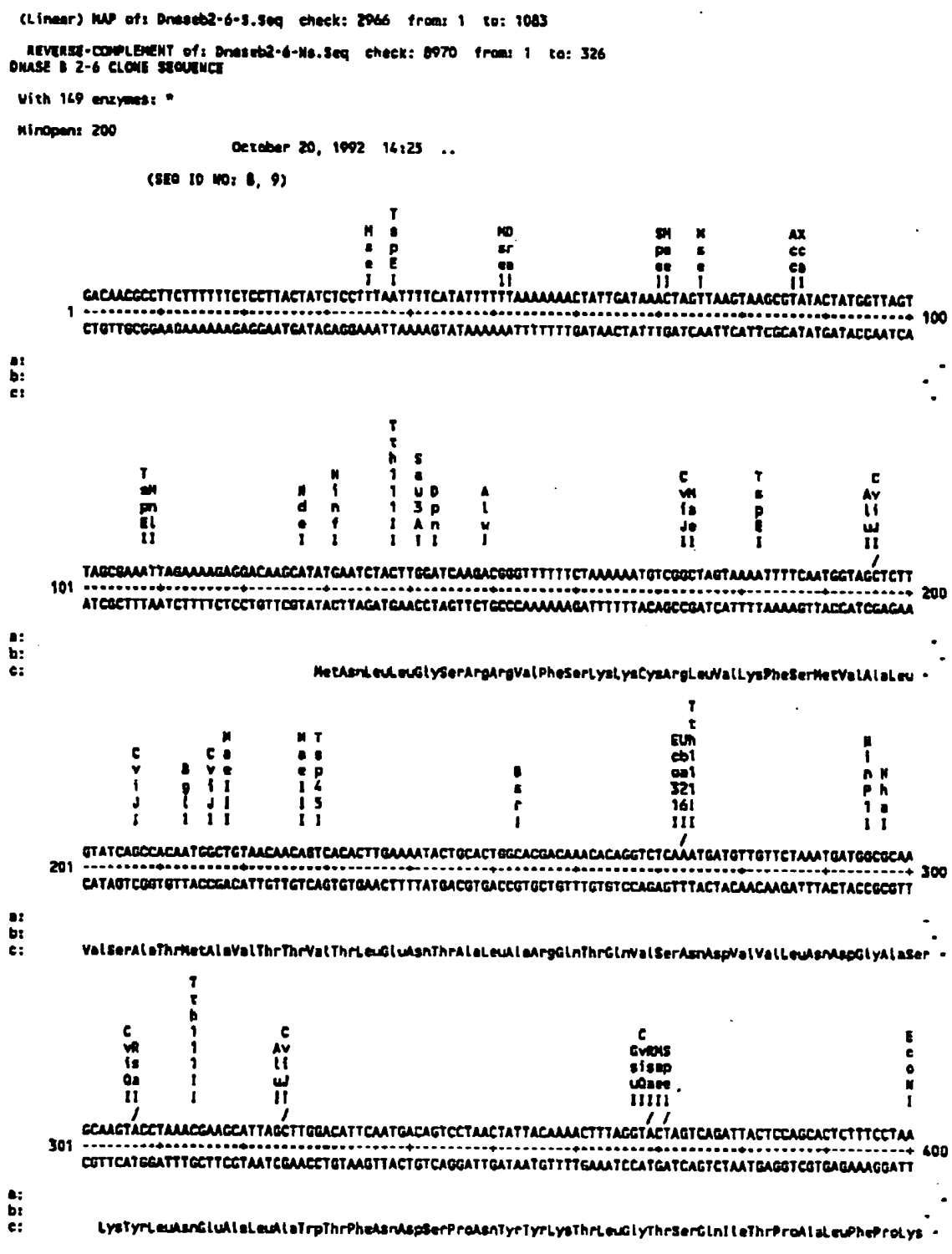

(Linear) MAP of: Dnaseb2-6-S.Seq check: 2966 from: 1 to: 1083
REVERSE-COMPLEMENT of: Dnaseb2-6-Ns.Seq check: 8970 from: 1 to: 326
DNASE B 2-6 CLONE SEQUENCE
With 169 enzymes: *
MinOpen: 200
October 20, 1992 14:25 ..
(SEQ ID NO: 8, 9)
GACAACGCCTTCTTTTTTCTCCTTACTATCTCCTTTAATTTTCATATTTTTTAAAAAAACTATTGATAAACTAGTTAAGTAAGCGTATACTATGGTTAGT
CTGTTGCGGAAGAAAAAAGAGGAATGATAGAGGAAATTAAAAGTATAAAAAATTTTTTTGATAACTATTTGATCAATTCATTCGCATATGATACCAATCA
TAGCGAAATTAGAAAAAGAGGACAAGCATATGAATCTACTTGGATCAAGACGGGTTTTTTCTAAAAAATGTCGGCTAGTAAAATTTTCAATGGTAGCTCTT
ATCGCTTTAATCTTTTCTCCTGTTCGTATACTTAGATGAACCTAGTTCTGCCCAAAAAAGATTTTTTACAGCCGATCATTTTAAAAGTTACCATCGAGAA
MetAsnLeuLeuGlySerArgArgValPheSerLysLysCysArgLeuValLysPheSerMetValAlaLeu
GTATCAGCCACAATGGCTGTAACAACAGTCACACTTGAAAATACTGCACTGGCACGACAAACACAGGTCTCAAATGATGTTGTTCTAAATGATGGCGCAA
CATAGTCGGTGTTACCGACATTGTTGTCAGTGTGAACTTTTATGACGTGACCGTGCTGTTTGTGTCCAGAGTTTACTACAACAAGATTTACTACCGCGTT
ValSerAlaThrMetAlaValThrThrValThrLeuGluAsnThrAlaLeuAlaArgGlnThrGlnValSerAsnAspValValLeuAsnAspGlyAlaSer
GCAAGTACCTAAACGAAGCATTAGCTTGGACATTCAATGACAGTCCTAACTATTACAAAACTTTAGGTACTAGTCAGATTACTCCAGCACTCTTTCCTAA
CGTTCATGGATTTGCTTCGTAATCGAACCTGTAAGTTACTGTCAGGATTGATAATGTTTTGAAATCCATGATCAGTCTAATGAGGTCGTGAGAAAGGATT
LysTyrLeuAsnGluAlaLeuAlaTrpThrPheAsnAspSerProAsnTyrTyrLysThrLeuGlyThrSerGlnIleThrProAlaLeuPheProLys

FIG. 3

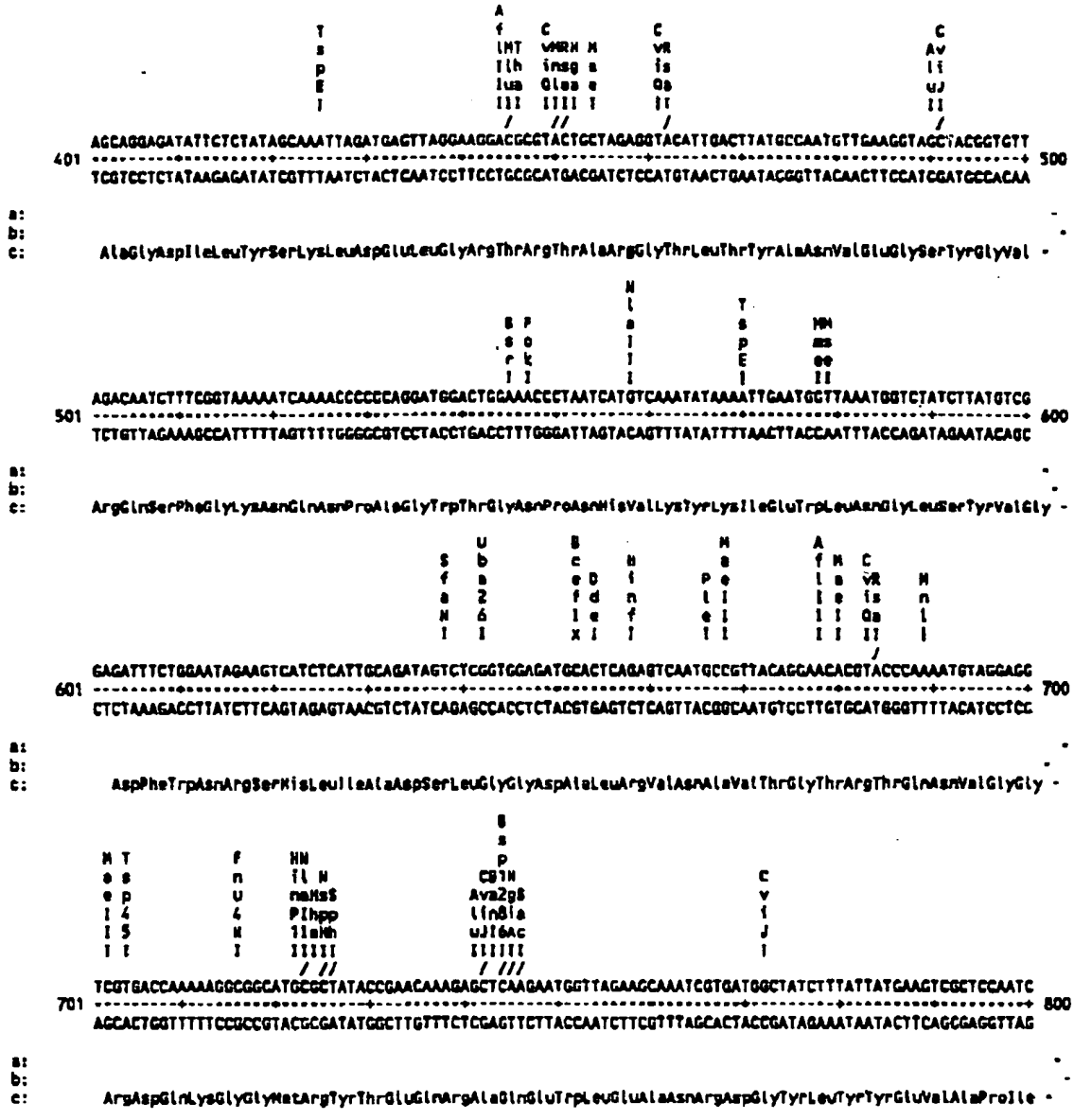


FIG 3, cont.

```
                   H         C         H                                                  C
                   i         Av        b                                                  Av
                   n         li        o                                                  li
                   f         uJ        I                                                  uJ
                   I         II        I                                                  II
                             /                                                            /
     TACAACGCAGACGAGTTGATTCCAAGAGCTGTCGTGGTATCAATGCAATCTTCTGATAATACCATCAACGAGAAAGTATTAGTTTACAACACAGCTAATG
 801 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 900
     ATGTTGCGTCTGCTCAACTAAGGTTCTCGACAGCACCATAGTTACGTTAGAAGACTATTATGGTAGTTGCTCTTTCATAATCAAATGTTGTGTCGATTAC

a:                                                                                                        -
b:                                                                                                        .
c:   TyrAsnAlaAspGluLeuIleProArgAlaValValValSerMetGlnSerSerAspAsnThrIleAsnGluLysValLeuValTyrAsnThrAlaAsnGly -

                                                                              H
     C      H                C                                    C         C a  C
     v      s                vE                                   vH     HH vHaMSAv
     i      e                is                                   ia     an ialatli
     J      I                Qa                                   Je     el JeleuuJ
     I                       II                                   II     II IIIIIII
                             /                                           /  //// /
     GCTACACCATTAACTACCATAACGGTACACCTACTCAAAAATAATACCAAAAGGCTAGACCTCTGCTCACTAGGCCTAGCTTTTTACATCAAAAAAAGCA
 901 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 1000
     CGATGTGGTAATTGATGGTATTGCCATGTGGATGAGTTTTTATTATGGTTTTCCGATCTGGAGACGAGTGATCCGGATCGAAAAATGTAGTTTTTTTCGT

a:                                                                                                        -
b:                                                                                                        .
c:     TyrThrIleAsnTyrHisAsnGlyThrProThrGlnLysEnd                                                         .

                              B
                              c                                T
                           H  e                                s
                           a  f                                p
                           e  I                                E
                           I  x                                I
     ATGACTATAGAAAGTAAAAATACTAGAAAAAGCAATGATTGCCGTCATTGCTTTTTATGAATTTGTGCAAAAAGCAAAAAAGC
1001 ---------+---------+---------+---------+---------+---------+---------+---------+--- 1083
     TACTGATATCTTTCATTTTTATGATCTTTTTCGTTACTAACGGCAGTAACGAAAAATACTTAAACACGTTTTTCGTTTTTTCG

a:                                                                   MetAsnLeuCysLysLysGlnLysSer -
b:                                                                                              .
c:                                                 MetIleAlaValIleAlaPheTyrGluPheValGlnLysAlaLysLys??? -
```

Enzymes that do cut:

AccI	AflIII	AluI	AluI	BanII	Bcefix	BglI	Bsp1286I	BsrI	CviJI	CviQI	DdeI	DpnI
DraI	Eco31I	EcoNI	Fnu4HI	FokI	GsuI	HaeI	HaeIII	HgaI	HgiAI	HhaI	HinfI	HinP1I
MaeI	MaeII	MaeIII	MboII	MluI	MmeI	MnlI	NseI	NdeI	NlaIII	NspHI	PleI	RsaI
SacI	Sau3AI	SfaNI	SpeI	SphI	StuI	ThaI	Tsp45I	TspEI	Tth111II	Ube26I	XcaI	

Enzymes that do not cut:

AatII	AflII	AhaII	AlwNI	AocI	ApaI	ApaLI	Asp700I	Asp718I	AsuII	AvaI	AvaII	AvrII
BalI	BamHI	BanI	BbeI	BbvI	BbvII	BclI	BglII	BsmI	BspHI	BspMI	BspMII	BssHII
BstEII	BstNI	BstXI	CfrI	Cfr10I	ClaI	DraII	DraIII	DsaI	Eco47III	Eco57I	Eco78I	EcoRI
EcoRII	EcoRV	EspI	FinI	FinI	FspI	GdiII	HaeII	HgiEII	HincII	HindIII	HpaI	HpaII
HphI	KpnI	Ksp632I	MfeI	NaeI	NarI	NciI	NcoI	NheI	NlaIV	NotI	NruI	MslI
NspBII	PflMI	PmaCI	PpuMI	PssI	PstI	PvuI	PvuII	RsrII	SacII	SalI	Sau96I	ScaI
ScII	ScrFI	SecI	SfiI	SmaI	SnaBI	SplI	SsoII	SspI	StyI	TaqI	TaqII	TaqII
Tth111I	VspI	XbaI	XhoI	XhoII	XmaI	XmaIII						

FIG. 3, cont

FIGURE 4

AMINO ACID SEQUENCE OF CLONED S. pyogenes DNase

R-Q-T-Q-V-S-N-D-V-V-L-N-D-G-A-S-K-Y-L-N-
E-A-L-A-W-T-F-N-D-S-P-N-Y-Y-K-T-L-G-T-S-
Q-I-T-P-A-L-F-P-K-A-G-D-I-L-Y-S-K-L-D-E-
L-G-R-T-R-T-A-R-G-T-L-T-Y-A-N-V-E-G-S-Y-
G-V-R-Q-S-F-G-K-N-Q-N-P-A-G-W-T-G-N-P-N-
H-V-K-Y-K-I-E-W-L-N-G-L-S-Y-V-G-D-F-W-N-
R-S-H-L-I-A-D-S-L-G-G-D-A-L-R-V-N-A-V-T-
G-T-R-T-Q-N-V-G-G-R-D-Q-K-G-G-M-R-Y-T-E-
Q-R-A-Q-E-W-L-E-A-N-R-D-G-Y-L-Y-Y-E-V-A-
P-I-Y-N-A-D-E-L-I-P-R-A-V-V-V-S-M-Q-S-S-
D-N-T-I-N-E-K-V-L-V-Y-N-T-A-N-G-Y-T-I-N-
Y-H-N-G-T-P-T-Q-K

(SEQ ID NO: 9)

PCR OLIGONUCLEOTIDE

5' TAACGGATCCGAATCTACTTGGATCAAGACGGGTTTTTTCT 3' (SEQ ID No. 2)

```
    1 ATGGATCCGAATCTACTTGGATCAAGACGGGTTTTTTCTAAAAAATGTCGGCTAGTAAAA
      ·····+·····+·····+·····+·····+·····+·····+·····+·····+·····+ 60
      TACCTAGGCTTAGATGAACCTAGTTCTGCCCAAAAAAGATTTTTTACAGCCGATCATTTT

      MetAspProAsnLeuLeuGlySerArgArgValPheSerLysLysCysArgLeuValLys -

   61 TTTTCAATGGTAGCTCTTGTATCAGCCACAATGGCTGTAACAACAGTCACACTTGAAAAT
      ·····+·····+·····+·····+·····+·····+·····+·····+·····+·····+ 120
      AAAAGTTACCATCGAGAACATAGTCGGTGTTACCGACATTGTTGTCAGTGTGAACTTTTA

      PheSerMetValAlaLeuValSerAlaThrMetAlaValThrThrValThrLeuGluAsn -

  121 ACTGCACTGGCACGACAAACACAGGTCTCAAATGATGTTGTTCTAAATGATGGCGCAAGC
      ·····+·····+·····+·····+·····+·····+·····+·····+·····+·····+ 180
      TGACGTGACCGTGCTGTTTGTGTCCAGAGTTTACTACAACAAGATTTACTACCGCGTTCG

      ThrAlaLeuAlaArgGlnThrGlnValSerAsnAspValValLeuAsnAspGlyAlaSer -

  181 AAGTACCTAAACGAAGCATTAGCTTGGACATTCAATGACAGTCCTAACTATTACAAAACT
      ·····+·····+·····+·····+·····+·····+·····+·····+·····+·····+ 240
      TTCATGGATTTGCTTCGTAATCGAACCTGTAAGTTACTGTCAGGATTGATAATGTTTTGA

      LysTyrLeuAsnGluAlaLeuAlaTrpThrPheAsnAspSerProAsnTyrTyrLysThr -

  241 TTAGGTACTAGTCAGATTACTCCAGCACTCTTTCCTAAAGCAGGAGATATTCTCTATAGC
      ·····+·····+·····+·····+·····+·····+·····+·····+·····+·····+ 300
      AATCCATGATCAGTCTAATGAGGTCGTGAGAAAGGATTTCGTCCTCTATAAGAGATATCG

      LeuGlyThrSerGlnIleThrProAlaLeuPheProLysAlaGlyAspIleLeuTyrSer -

  301 AAATTAGATGAGTTAGGAAGGACGCGTACTGCTAGAGGTACATTGACTTATGCCAATGTT
      ·····+·····+·····+·····+·····+·····+·····+·····+·····+·····+ 360
      TTTAATCTACTCAATCCTTCCTGCGCATGACGATCTCCATGTAACTGAATACGGTTACAA

      LysLeuAspGluLeuGlyArgThrArgThrAlaArgGlyThrLeuThrTyrAlaAsnVal -

  361 GAAGGTAGCTACGGTGTTAGACAATCTTTCGGTAAAAATCAAAACCCCGCAGGATGGACT
      ·····+·····+·····+·····+·····+·····+·····+·····+·····+·····+ 420
      CTTCCATCGATGCCACAATCTGTTAGAAAGCCATTTTTAGTTTTGGGGCGTCCTACCTGA

      GluGlySerTyrGlyValArgGlnSerPheGlyLysAsnGlnAsnProAlaGlyTrpThr -

  421 GGAAACCCTAATCATGTCAAATATAAAATTGAATGGTTAAATGGTCTATCTTATGTCGGA
      ·····+·····+·····+·····+·····+·····+·····+·····+·····+·····+ 480
      CCTTTGGGATTAGTACAGTTTATATTTTAACTTACCAATTTACCAGATAGAATACAGCCT

      GlyAsnProAsnHisValLysTyrLysIleGluTrpLeuAsnGlyLeuSerTyrValGly -
```

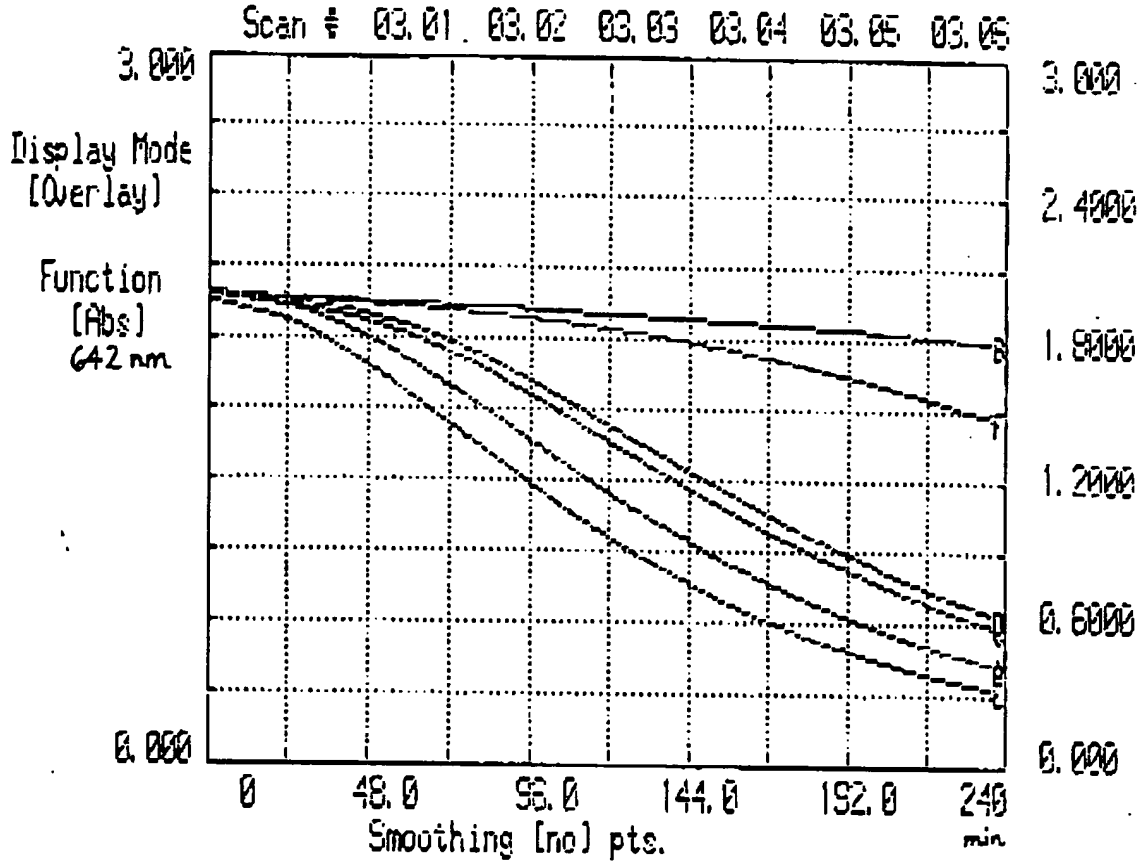
FIG. 5

461 ... 540 (SEQ ID No: 11) 944

3' TCTTTTTCGTTACTAACGGCAGTAACGGGGCCCAGCTGGGCC 5' (SEQ ID No: 3)

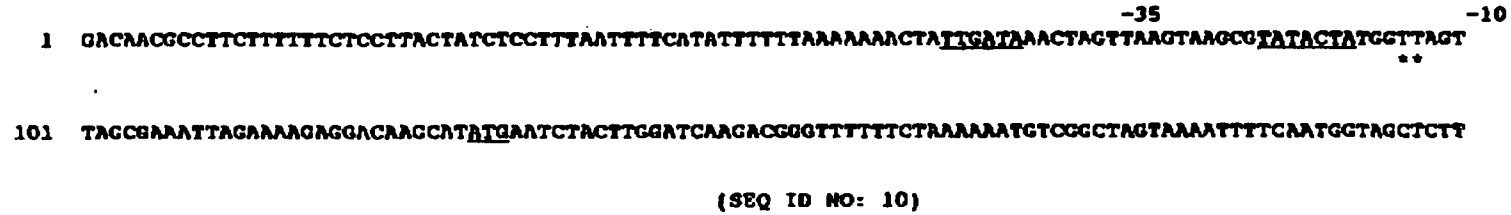
PCR OLIGONUCLEOTIDE

FIG. 5, cont.



A = Streptonase B
B = Streptonase B + Antibody
C = DNaseI-1
D = DNaseI-1 + Antibody
E = DNase B2-6
F = DNase B2-6 + Antibody

FIG. 6



(SEQ ID NO: 10)

Consensus sequence of Escherichia coli promoter region:

-35	-10
tcTTGACat	TAtAaT

FIG. 7

Correlation Curve of Activity Assays Based On Recombinant and Natural (Streptonase B) DNAase B

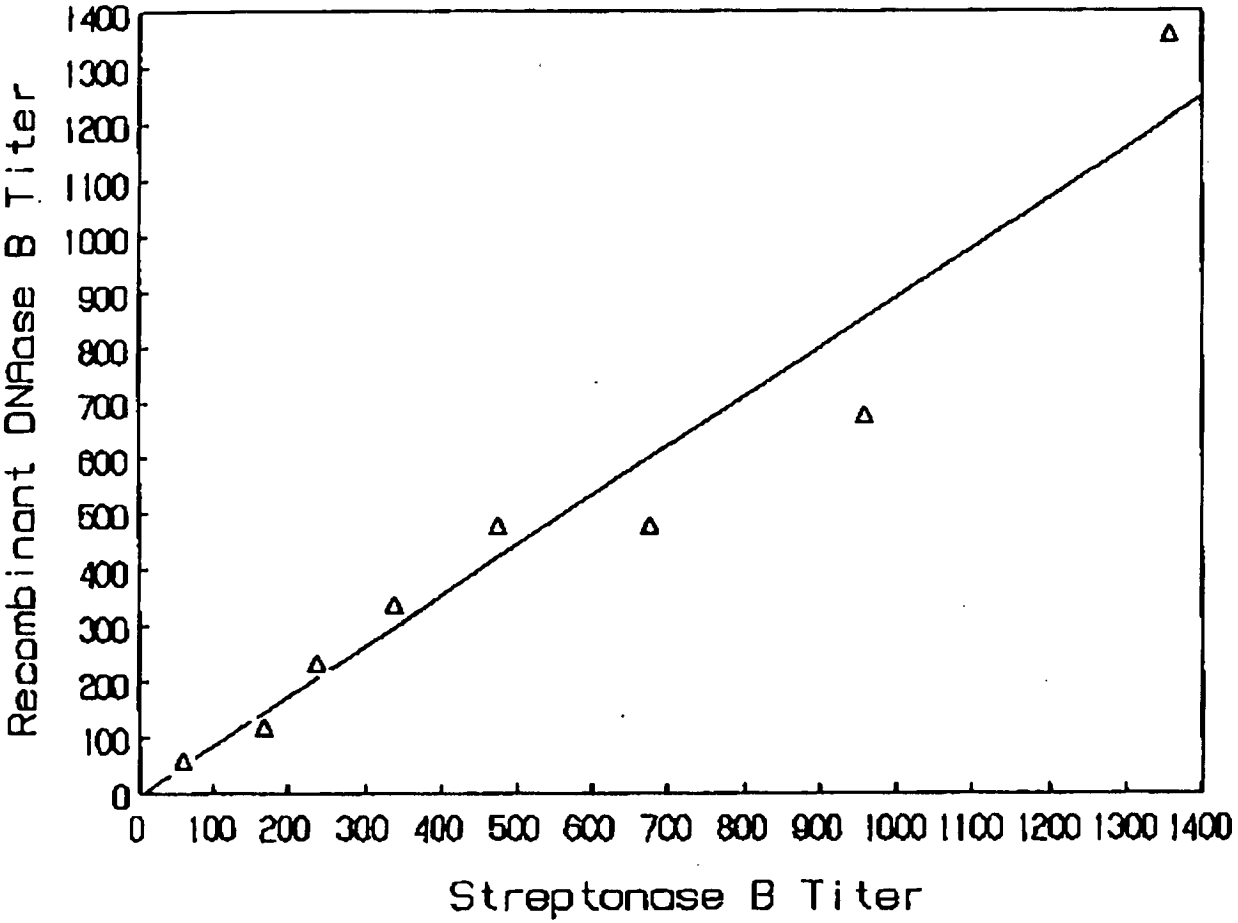


FIG 8

MITOGENIC ASSAY WITH MOUSE LYMPHOCYTES

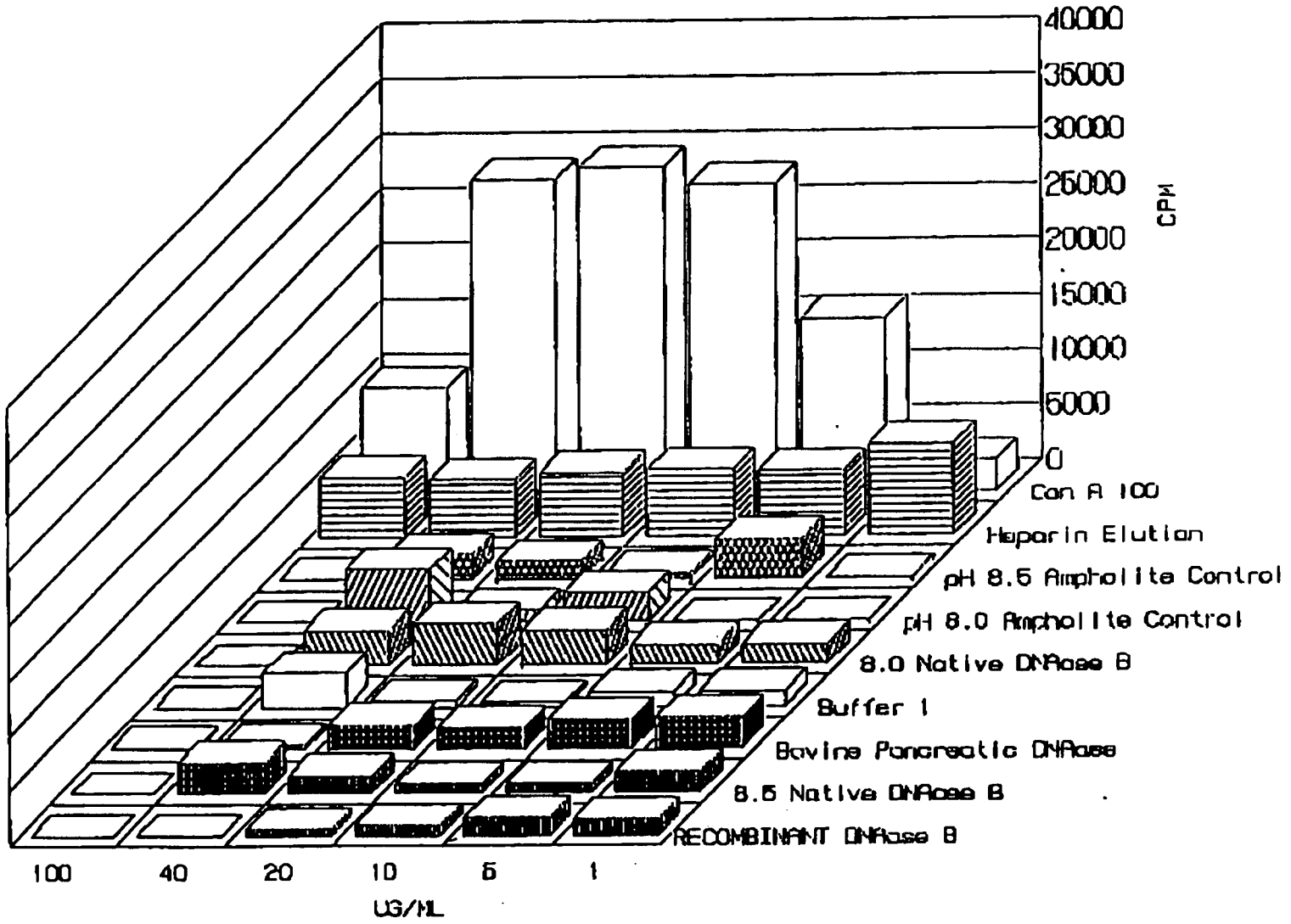


FIG 9

SEQUENCE OF CONSTRUCTION PRODUCING DNASE B
PROCESSED IDENTICALLY TO NATURAL DNASE B

PCR OLIGONUCLEOTIDE (SEQ ID NO: 12)

```
5'AGGCAATGGATCCGAACCTGCTGGGTTCCCGTCGTGTTTTCTCCAAAAAATGCCGTCTGGTTAAATTCTCCAT

      ATGGATCCGAACCTGCTGGGTTCCCGTCGTGTTTTCTCCAAAAAATGCCGTCTGGTTAAATTCTCCAT
            1 ---------+---------+---------+---------+---------+---------+ 60
      TACCTAGGCTTAGATGAACCTAGTTCTGCCCAAAAAAGATTTTTTACAGCCGATCATTTTAAAAGTTA

      MetAspProAsnLeuLeuGlySerArgArgValPheSerLysLysCysArgLeuValLysPheSerMet -

          GGTTGCTCTGGTTTCCGCTACCATGGCTGTTACCACCGTTACCCTGGAAAACACCGCTCT

          GGTTGCTCTGGTTTCCGCTACCATGGCTGTTACCACCGTTACCCTGGAAAACACCGCTCT
       61 ---------+---------+---------+---------+---------+---------+ 120
          CCATCGAGAACATAGTCGGTGTTACCGACATTGTTGTCAGTGTGAACTTTTATGACGTGA

           ValAlaLeuValSerAlaThrMetAlaValThrThrValThrLeuGluAsnThrAlaLeu -

          GGCT***CAGACACAGGTCTCAAATGATGTTGTTCTAAATGATGGCGCAAGCTTCATGGA

          GGCT***CAGACACAGGTCTCAAATGATGTTGTTCTAAATGATGGCGCAAGCAAGTACCT
      121 ---------+---------+---------+---------+---------+---------+ 180
          CCGTGCTGTTTGTGTCCAGAGTTTACTACAACAAGATTTACTACCGCGTTCGTTCATGGA

           AlaArgGlnThrGlnValSerAsnAspValValLeuAsnAspGlyAlaSerLysTyrLeu -

          AAACGAAGCATTAGCTTGGACATTCAATGACAGTCCTAACTATTACAAAACTTTAGGTAC
      181 ---------+---------+---------+---------+---------+---------+ 240
          TTTGCTTCGTAATCGAACCTGTAAGTTACTGTCAGGATTGATAATGTTTTGAAATCCATG

           AsnGluAlaLeuAlaTrpThrPheAsnAspSerProAsnTyrTyrLysThrLeuGlyThr -

          TAGTCAGATTACTCCAGCACTCTTTCCTAAAGCAGGAGATATTCTCTATAGCAAATTAGA
      241 ---------+---------+---------+---------+---------+---------+ 300
          ATCAGTCTAATGAGGTCGTGAGAAAGGATTTCGTCCTCTATAAGAGATATCGTTTAATCT

           SerGlnIleThrProAlaLeuPheProLysAlaGlyAspIleLeuTyrSerLysLeuAsp -

          TGAGTTACGAAGGACGCGTACTGCTAGAGGTACATTGACTTATGCCAATGTTGAAGGTAG
      301 ---------+---------+---------+---------+---------+---------+ 360
          ACTCAATCCTTCCTGCGCATGACGATCTCCATGTAACTGAATACGGTTACAACTTCCATC

           GluLeuGlyArgThrArgThrAlaArgGlyThrLeuThrTyrAlaAsnValGluGlySer -

          CTACGGTGTTAGACAATCTTTCGCTAAAAATCAAAACCCCGCAGGATGGACTGCAAACCC
      361 ---------+---------+---------+---------+---------+---------+ 420
          GATGCCACAATCTGTTAGAAAGCCATTTTTAGTTTTGGGGCGTCCTACCTGACCTTTGGG

           TyrGlyValArgGlnSerPheGlyLysAsnGlnAsnProAlaGlyTrpThrGlyAsnPro -

          TAATCATGTCAAATATAAAATTGAATGGTTAAATGGTCTATCTTATGTCGGAGATTTCTG
      421 ---------+---------+---------+---------+---------+---------+ 480
          ATTAGTACAGTTTATATTTTAACTTACCAATTTACCAGATAGAATACAGCCTCTAAAGAC

           AsnHisValLysTyrLysIleGluTrpLeuAsnGlyLeuSerTyrValGlyAspPheTrp -

          GAATAGAAGTCATCTCATTGCAGATAGTCTCGGTGGAGATGCACTCAGAGTCAATGCCGT
      481 ---------+---------+---------+---------+---------+---------+ 540
          CTTATCTTCAGTAGAGTAACGTCTATCAGAGCCACCTCTACGTGAGTCTCAGTTACGGCA

           AsnArgSerHisLeuIleAlaAspSerLeuGlyGlyAspAlaLeuArgValAsnAlaVal -
```

FIG. 10

```
    TACAGGAACACGTACCCAAAATGTAGGAGGTCGTGACCAAAAAGGCGGCATGCGCTATAC
541 ----------+---------+---------+---------+---------+---------+ 600
    ATGTCCTTGTGCATGGGTTTTACATCCTCCAGCACTGGTTTTTCCGCCGTACGCGATATG

     ThrGlyThrArgThrGlnAsnValGlyGlyArgAspGlnLysGlyGlyMetArgTyrThr -

    CGAACAAAGAGCTCAAGAATGGTTAGAAGCAAATCGTGATGGCTATCTTTATTATGAAGT
601 ----------+---------+---------+---------+---------+---------+ 660
    GCTTGTTTCTCGAGTTCTTACCAATCTTCGTTTAGCACTACCGATAGAAATAATACTTCA

     GluGlnArgAlaGlnGluTrpLeuGluAlaAsnArgAspGlyTyrLeuTyrTyrGluVal -

    CGCTCCAATCTACAACGCAGACGAGTTGATTCCAAGAGCTGTCGTGGTATCAATGCAATC
661 ----------+---------+---------+---------+---------+---------+ 720
    GCGAGGTTAGATGTTGCGTCTGCTCAACTAAGGTTCTCGACAGCACCATAGTTACGTTAG

     AlaProIleTyrAsnAlaAspGluLeuIleProArgAlaValValValSerMetGlnSer -

    TTCTGATAATACCATCAACGAGAAAGTATTAGTTTACAACACAGCTAATGGCTACACCAT
721 ----------+---------+---------+---------+---------+---------+ 780
    AAGACTATTATGGTAGTTGCTCTTTCATAATCAAATGTTGTGTCGATTACCGATGTGGTA

     SerAspAsnThrIleAsnGluLysValLeuValTyrAsnThrAlaAsnGlyTyrThrIle -

    TAACTACCATAACGGTACACCTACTCAAAAATAATACCAAAAGGCTAGACCTCTGCTCAC
781 ----------+---------+---------+---------+---------+---------+ 840
    ATTGATGGTATTGCCATGTGGATGAGTTTTTATTATGGTTTTCCGATCTGGAGACGAGTG

     AsnTyrHisAsnGlyThrProThrGlnLysEnd  (SEQ ID NO: 15)

    TAGGCCTAGCTTTTTACATCAAAAAAAGCAATGACTATAGAAAGTAAAAATACTAGAAAA
841 ----------+---------+---------+---------+---------+---------+ 900
    ATCCGGATCGAAAAATGTAGTTTTTTTCGTTACTGATATCTTTCATTTTTATGATCTTTT

                                                          3' TCTTTT

    AGCAATGATTGCCGTCATTGCCCCGGGTCGAC (SEQ ID NO: 14)
901 ---------+---------+---------+------ 936
    TCGTTACTAACGGCAGTAACGGGGCCCAGCTG

    TCGTTACTAACGGCAGTAACGGGGCCCAGCTGGGCC 5' (SEQ ID NO: 13)
       PCR OLIGONUCLEOTIDE
```

FIG. 10, Cont.